Basic Cytogenetics Laboratory Procedures

The study of chromosomes using traditional cytogenetic techniques requires cells that are actively dividing Chromosomes are individually distinguishable under the light microscope only during cell division and are best examined during metaphase.

Specimens that contain spontaneously proliferating cells include :

- bone marrow
- lymph nodes
- solid tumors tissue biopsies
- amniotic fluids
- Chorionic villi

Specimen Collection and Handling

- **Peripheral Blood Specimens**

Peripheral blood samples should be collected in sterile syringes or vacuum tubes containing preservative-free *sodium* heparin for best results, blood samples should be set up within 24 h of collection Temperature extremes must be avoided if samples are transported or stored.

Specimens should be kept at room *temperature* or refrigerated above 4°C until they can be processed a repeat sample should be requested if these requirements are not met (e.g., the sample is received clotted, on ice, more than 24 h old)

Bone marrow aspirates

- should be collected in sterile syringes or vacuum tubes containing preservative-free *sodium* heparin
- transported at room temperature
- The first few milliliters of the bone marrow tap contain the highest proportion of cells are the best sample for the cytogenetics laboratory.

Bone marrow specimens should be processed immediately upon receipt to avoid cell death.

Amniotic Fluid Specimens

- can be performed from as early as 10 weeks of gestation until term
- 15 to 30 milliliter of amniotic fluid should be obtained under sterile conditions and collected in a sterile container approved for cell culture.
- Samples should be transported at room temperature. Temperature extremes and long transport times should be avoided the amniocentesis procedure has an inherent, albeit small, risk of miscarriage and should not be repeated unless absolutely necessary

Solid Tissue Biopsies

- Solid tissue sources include
- skin biopsies
- chorionic villi
- products of conception

- lymph node and solid tumor biopsies
- tissue from stillbirths

Products of conception and stillbirths (and in most cases, tumor biopsies) are one-of-a-kind specimens that cannot be recollected, and repeat collection of chorionic villi increases the risk of miscarriage, although subsequent amniocentesis is an option here.

Microbial contamination is a common problem for many types of solid tissue samples.

Culture Initiation

Growth Media

- AmnioMAX™, Chang Medium or Amniochrome for amniocytes
- giant cell tumor-conditioned medium for malignancies
- PANDIS for breast tumors while others are appropriate for a broad spectrum of cell types (e.g., RPMI 1640, MEM)
- All culture media are balanced salt solutions with a variety of additives including salts, glucose, and a buffering system to maintain the proper pH.
- Phenol red is often used as a pH indicator in many media.

If the medium becomes too acidic, it will turn yellow, while medium that is too basic becomes pink or purple.

L -Glutamine

l -Glutamine is an amino acid essential for cell growth.

l -Glutamine is unstable and breaks down on storage to d -glutamine, a form that cannot be used by cells. l -Glutamine must therefore be stored frozen to retain its stability, and it is optimal to add it to the culture

medium just prior to use. There are some commercially available complete media that contain l -glutamine.

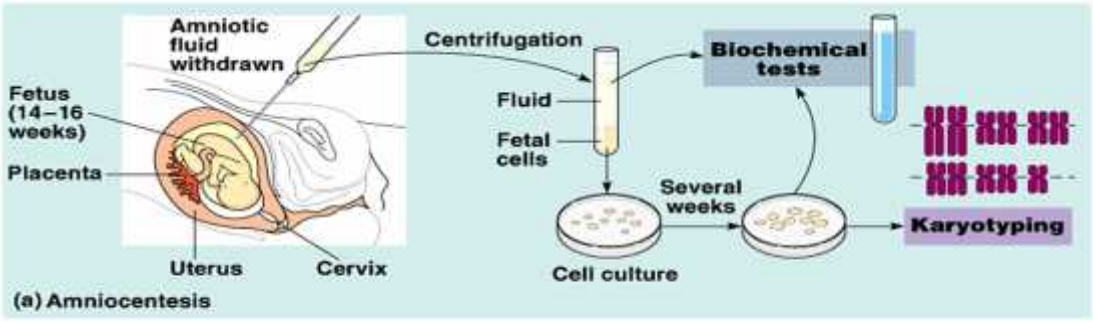
Serum

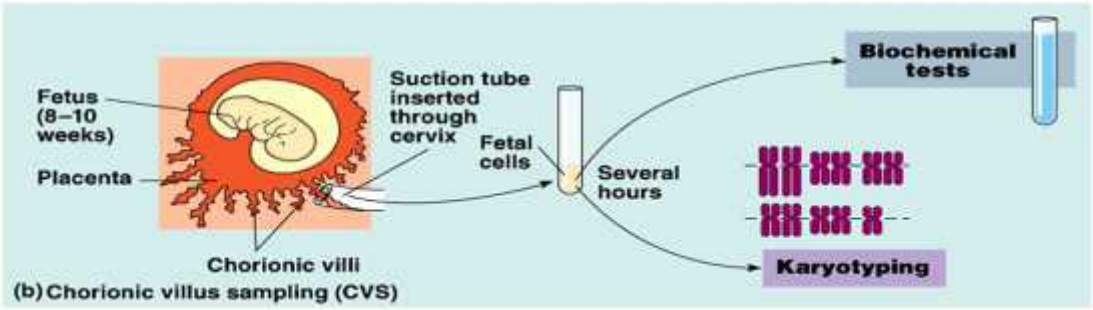
- Serum is essential for good cell growth.
- Too little does not allow for maximum cell growth, but too much can have a detrimental effect.
- Fetal bovine serum (FBS) is preferred; culture medium is generally supplemented with 10–30% FBS.

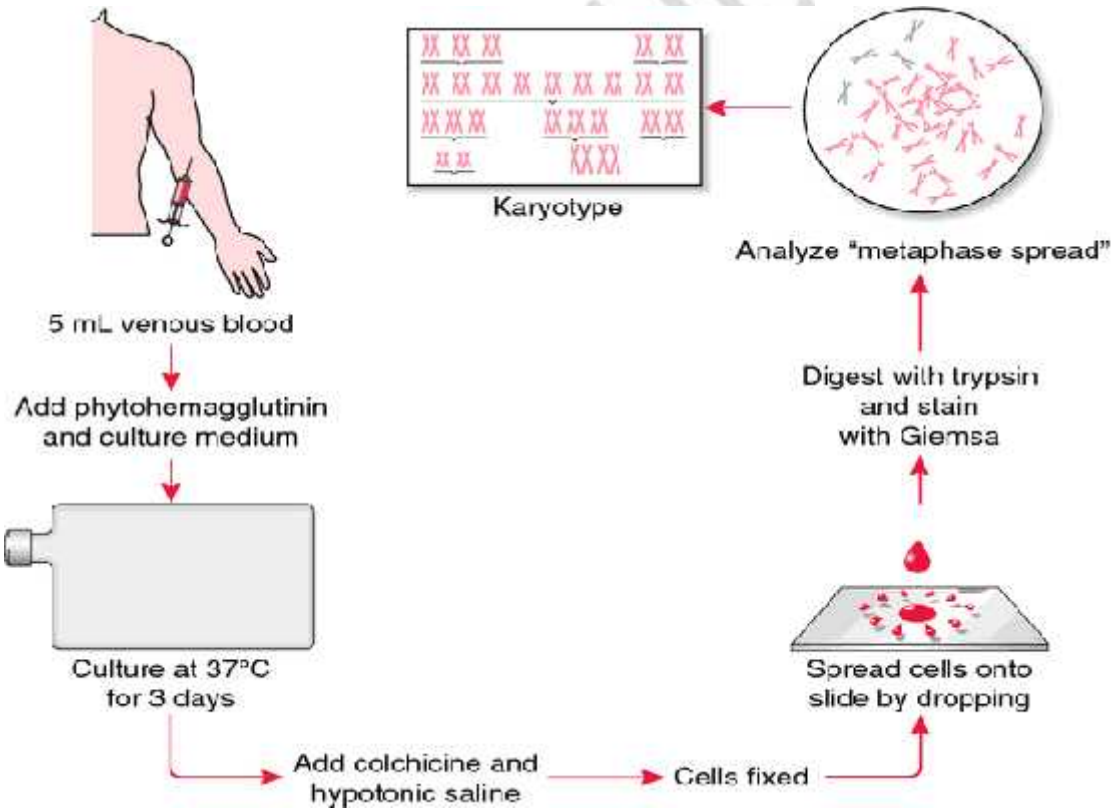
Antibiotics

- Microbial inhibitors are added to culture media to retard the growth of microorganisms Penicillin/streptomycin, kanamycin, and gentamicin are bacterial inhibitors commonly used in tissue culture

- **Mitotic Stimulants (Mitogens)**
- Some cells, particularly mature lymphocytes, do not spontaneously undergo cell division and must be stimulated to divide by the addition of an appropriate mitogen to the cell culture.
- Phytohemagglutinin (PHA) is an extract of red kidney beans that stimulates division primarily of T-lymphocytes
- For routine peripheral blood cultures, 72 h is usually optimal


Amniotic fluid withdrawn
Centrifugation
Biochemical tests
Fetus (14–16 weeks)
Placenta
Fluid
Fetal cells
Several weeks
Karyotyping
Uterus
Cervix
Cell culture
(a) Amniocentesis


Biochemical tests
Fetus (8–10 weeks)
Placenta
Suction tube inserted through cervix
Fetal cells
Several hours
Karyotyping
Chorionic villi
(b) Chorionic villus sampling (CVS)


5 mL venous blood
Add phytohemagglutinin and culture medium
Culture at 37°C for 3 days
Add colchicine and hypotonic saline
Cells fixed
Spread cells onto slide by dropping
Digest with trypsin and stain with Giemsa
Analyze "metaphase spread"
Karyotype